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The Diversity and Antagonistic Ability of *Trichoderma* spp. on the *Aspergillus Flavus* Pathogen on Peanuts in North Center of Vietnam

Nguyen Thi Thanh[1,*], Ho Thi Nhung[1], Nguyen Thi Thuy[1], Thai Thi Ngoc Lam[1], Phan Thi Giang[1], Tran Ngoc Lan[2], Nguyen Van Viet[3], Vu Trieu Man[4]

[1]Vinh University, Vinh, Vietnam
[2]Institute of Regional Research and Development, Ha Noi, Vietnam
[3]Vietnam Academy of Agricultural Sciences, Ha Noi, Vietnam
[4]Phytopathological Society of Vietnam, Research Centre for Plant and Animal Health, Hanoi, Vietnam
*Corresponding author: phuongthanh1908@gmail.com

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Abstract Among 1000 peanut soil samples were collected from Nghe An province, Thanh Hoa province and Ha Tinh province, 44.90% samples occurred *Trichoderma*. The appearance ratio of *Trichoderma* in Nghe An, Thanh Hoa, Ha Tinh is 44.30%, 52.00% and 39.50%, respectively. By using in the dual culture method on PDA, The Percent Inhibition of Mycelial Growth (PIRG) was determined to be very high in 28 *Trichoderma* strains (made up 28.3% in total), 29 others strains was dertermined that to be high PIRG (made up 29,3% in total). The highest PIRG was 100%, occurred on *T. harzianum* (*Tri*.019(4).NC; *Tri*.053(1).TG; *Tri*.011(1).NL; *Tri*.002(2).NX strain), *T. atroviride* (*Tri*.020(2).NC; *Tri*.011(1).NC strain), *T. reesci* (*Tri*.007(1).NĐ), *T. hamatum* (*Tri*.039(1).TG), *T. virens* (*Tri*.014(4).NC) and *T. pseudokonigii* (*Tri*.014(1).NL).

Keywords: *Antagonistic ability, Aspergillus flavus, Diversity, PIRG, Trichoderma*

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1. Introduction

In Vietnam, peanut (*Arachis hypogaea* L.) is known as a short- day crop of high economic value, to be used as food and for export. Peanut contains 50% lipid, 22-25 % protein, and also contains 8 essential amino acids and soluble vitamins such as B1 (Thiamine), B2 (Riboflavin), PP (nicotinic oxide), E F ... (Nguyen Van Binh et al.). Peanut was listed among top ten exported products of Vietnam. It was the second biggest exported crops after rice of Vietnam (Vietnam General Statistics Office, 2004). Simultaneously, peanut exportation reached 32000 – 35000 ton, ranked 5th out of ten biggest export countries of the world. In 2013, peanut cultivated surface of Vietnam was approximately 216300 ha, its production reached to 492600 ton.

North Center region of Vietnam, includes three provinces Thanh Hoa, Nghe An and Ha Tinh, has the largest cultivated surface of peanut, make up 23.30% in total cultivated surface and 22.91% of total production in whole country. Cultivated surfaces of Nghe An, Ha Tinh, Thanh Hoa were 19600 ha; 17300 ha; 13500 ha, made up 9.06%; 8.00%; 6.24% in total peanut cultivated surface of Vietnam, respectively. Peanut productions of these provinces were 44500 ton, 40800 ton, 27600 ton, respectively made up 9.03%; 8.28%; 5.60% in total production (Vietnam General Statistics Office, 2013).

Aspergillus flavus pathogen mainly infect peanut, produce toxic compound called aflatoxin, causes health risks like cancer and other dangerous diseases in human and animals. Many countries have attempted to limit exposure to aflatoxins by imposing regulatory limits on importation of peanut. To help minimize risk for human and animal health, many cultivate peanut countries, include Vietnam, have concerned in researching and controlling *A. flavus* fungi on peanut.

In recent years, researchers on the world tend to use biological control to prevent *Aspergillus flavus* fungi causing aflatoxin by three main ways: (i) Using Bacillus (*B. pumilus, B. subtilis*) (Munimbazi et al., 1998); (ii) Using nontoxigenic *Aspergillus flavus, A. Parasiticus* strains, that had successful application in US, like *Aspergillus flavus* AF36 (Cotty et al., 2004); (iii) Using antagonistic fungi *Trichoderma*, also had good result, such as using five antagonistic fungi species *T. harzianum, T. viride, T. auroviride, T. longibrachiatum, T. hamatum* on bio-control *Aspergillus flavus* causing aflatoxin in India (Mausam et al., 2007; Reddy et al., 2010).

In Vietnam, famers still mainly use cultivations practices and some storage methods based on their

experience to management aflatoxin produced by *A. flavus* fungi on peanut, such as control crop season, using lime, expose peanut under the sun to ensure the humidity less than 9%, and store peanut in cool and dry condition. Scientists have researched methods of bio-control and resistant variety production to prevent *A. flavus* fungi causing aflatoxin. In 2010, Nguyen Thuy Chau et al. had first success on coffee, maize and peanut in not only pre- but also post-harvest stage by using nontoxigenic *A. flavus* fungi. Up to now, in Vietnam we have determined at least thirty three species of *Trichoderma*. But the application of antagonistic fungi *Trichoderma* to management aflatoxin produced by *Aspergillus flavus* in agriculture is still not concerned.

Because of above reasons, we carry out the research: The diversity and antagonistic ability of *Trichoderma* spp. on the *Aspergillus flavus* pathogen on peanuts in north center of Vietnam.

2. Materials and Methods

2.1. Materials

- 1000 soil samples were collected from peanut cultivation regions of Nghe An, Thanh Hoa, Ha Tinh provinces, Vietnam.

- *Trichoderma* strains: Eight species of *Trichoderma* -*T. harzianum, T. atroviride, T. reesci, T. hamatum, T. virens, T. pseudokonigii, T. aureoviride, T. viride* were isolated from different soil samples, cultured on PDA (Potato Dextro Agar) medium. Five days old culture of *Trichoderma* was used for each experiment.

- Pathogen source: *Aspergillus flavus* (VAD006) was isolated from the infected peanut seeds and cultured on PDA medium. Seven days old culture of pathogen was used for each experiment.

- Culture mediums: Water Agar (GA), Potato Dextrose Agar (PDA).

2.2. Methods

2.2.1. Collection and Isolation

- Collecting soil samples was followed by Lester, 2009 [2].

- Isolating *Trichoderma* from the soil different was followed by Waksman (1952); Johnson *et al*., (1962).

Test the antagonistic ability of *Trichoderma* on *A. flavus*: Evaluation the antagonistic ability of *Trichoderma* on *A. flavus* was followed by Rahman, M. F. Begum and M. F. Alam. (2009). In this method, the 90mm diameter culture plates contain Potato Dextro Agar (PDA) medium were used. On the medium of dual control plate, *Trichoderma* (T) was placed 2 cm away from the edge of the petri plate. *A. flavus* (C) was similarly placed 2 cm away from the edge of the petri dish and on the opposite side with *Trichoderma* sample. On a control plate, *A. flavus* was placed with the same manner on a fresh PDA plate (Figure 1). All pairings were incubated at 30°C. Antagonistic ability was tested after 7 days of incubation by measuring the radius of the *A. flavus* colony in the direction of the antagonist colony (R_2) and the radius of the *A. flavus* colony in the control plate (R_1). All the data was processed by using the formula developed by Skidmore and Dickinson (1976) - Percentage inhibition of radial growth (PIRG) formla.

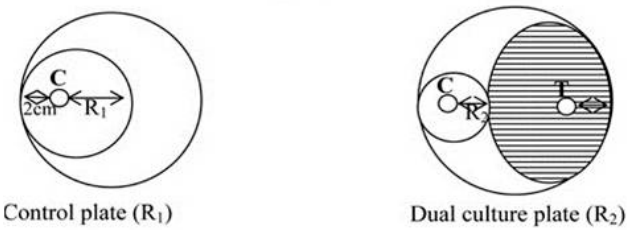

Figure 1. Measurement of radial growth of *A. flavus* mycelia by method where culture placement was 2 cm away from the margin. Note: R1, radius of *A. flavus* colony in control plate; R2, radius of A. *flavus* colony in dual culture plate; C, *A. flavus* isolate; T, *Trichoderma* isolate

Percent Inhibition ofRadialGrowth (PIRG):

$$\text{PIRG}\ (\%) = \frac{R1 - R2}{R1} \times 100$$

R1: The radius of *A. flavus* mycelium in the control plate

R2: The radius of *A. flavus* mycelium in the dual culture plate (*Trichoderma* and *A. flavus*).

The antagonistic levels:

PIRG ≤ 50%: Low

50% < PIRG ≤ 60%: Medium

60% < PIRG ≤ 75%: High

PIRG >75%: Very high

3. Results and Discussion

3.1. Collection, Isolation of *Trichoderma* Strains From the Soil Grows Peanuts in North Center Vietnam

Evaluation the occurrence frequency of the fungus *Trichoderma* over 1000 peanut soil samples in 3 representative provinces of the North center as showed in Table 1.

Table 1. The occurrence frequency of *Trichoderma* in North Center of Vietnam, 2011-2013

No.	Places	Total samples	Number of *Trichoderma* species	Number of *Trichoderma* strains	The appear frequency (%)
1	Nghe An	600	23	266	44.30
	Quynh Luu	200	7	99	49.50
	Dien Chau	200	8	110	55.00
	Nghi Loc	200	8	57	28.50
2	Ha Tinh	200	11	79	39.50
3	Thanh Hoa	200	8	104	52.00
	Total	1000	42	449	44.9

Through the collection of 1000 soil samples from three provinces, the appear frequency of *Trichoderma* strains was 44.90%. The appear frequency of *Trichoderma* strains of Nghe An, Thanh Hoa, Ha Tinh was 44.30%, 52.00% and 39.50%, respectively. In Nghe An province, the occurrence frequency of *Trichoderma* in Dien Chau

district was the highest with 55.00%, Quynh Luu district with 49.50% and the lowest was in Nghi Loc district with 28.50%.

3.2. Evaluating the Antagonistic Ability of *Trichoderma* spp. on *A. Flavus* Pathogen on Peanuts in North Center Vietnam

3.2.1. The Antagonistic Ability of *T. harzianum* on *A. flavus*

The antagonistic ability of 21 *T. harzianum* strains on *A. flavus* was shown at Table 2.

Table 2. The antagonistic ability of *T. harzianum* strains on *A. flavus* after 7 days of old fungal culture

No.	Provinces	Strains	PIRG (%)	The antagonistic levels
1		*Tri*.019(4).NC	100.00	++++
2		*Tri*.053(1).TG	100.00	++++
3		*Tri*.002(2).NC	95.71	++++
4		*Tri*.016(1).NC	74.32	+++
5	Thanh Hoa	*Tri*.012(2).NC	73.63	+++
6		*Tri*.034(1).TG	66.64	+++
7		*Tri*.014(1).NC	64.52	+++
8		*Tri*.049(2).TG	55.21	++
9		*Tri*.127(1).NC	45.79	+
10		*Tri*.127(2).NC	43.92	+
11		*Tri*.011(1).NL	100.00	++++
12	Nghe An	*Tri*.038(1).NL	70.00	+++
13		*Tri*.025(1).NĐ	68.00	+++
14		*Tri*.022(1).NL	58.80	++
15		*Tri*.028(2).NL	47.60	+
16		*Tri*.002(2).NX	100.00	++++
17		*Tri*.004(2).NX	94.51	++++
18	Ha Tinh	*Tri*.016(1).NX	65.26	+++
19		*Tri*.007(2).NA	62.70	+++
20		*Tri*.114(1).TH	26.71	+
21		*Tri*.092(2).TH	26.42	+

Very high (++++); High (+++); Medium (++); Low (+).

Among 21 *T. harzianum* strains, 6 strains were showed at very high level of antagonistic ability included *Tri*.019(4).NC, *Tri*.053(1).TG, *Tri*.002(2).NC, *Tri*.011(1).NL, *Tri*.002(2).NX and *Tri*.004(2).NX. There were 8 strains (38.09%) were showed at high antagonistic level.

3.2.2. The Antagonistic Ability of *T. atroviride* on *A. flavus*

The antagonistic ability of 18 strains of *T. atroviride* and *A. flavus* showed at Table 3.

Table 3. The antagonistic ability of *T. atroviride* on *A. flavus* after 7 days of old fungal culture

No.	Provinces	Strains	PIRG (%)	The antagonistic levels
1		*Tri*.020(2).NC	100.00	++++
2		*Tri*.011(1).NC	100.00	++++
3	Thanh Hoa	*Tri*.009(1).NC	83.40	++++
4		*Tri*.077(1).QX	70.37	+++
5		*Tri*.012(1).NC	61.61	+++
6		*Tri*.005(2).NĐ	89.31	++++
7		*Tri*.041(1).NĐ	85.98	++++
8		*Tri*.011(1).NĐ	65.60	+++
9	Nghe An	*Tri*.062(2).NĐ	45.21	+
10		*Tri*.008(1).NĐ	44.91	+
11		*Tri*.008(2).NĐ	42.46	+
12		*Tri*.010(1).NĐ	42.42	+
13		*Tri*.023(3).NP	5.00	+
14		*Tri*.005(1)NX	91.01	++++
15		*Tri*.008(1).NT	78.00	++++
16	Ha Tinh	*Tri*.038(2).NX	75.22	++++
17		*Tri*.032(1).NX	48.61	+
18		*Tri*.023(3).NP	5.00	+

Very high (++++); High (+++); Medium (++); Low (+).

Eight strains (44.44%) were showed at very high PIRG among 18 strains of *T. atroviride*, including *Tri*.020(2).NC, *Tri*.011(1).NC, *Tri*.005(2).NĐ, *Tri*.009(1).NC, *Tri*.041(1).NĐ, *Tri*.005(1).NX, *Tri*.008(1).NT and *Tri*.038(2).NX. There were 3 strains (16.67%) with PIRG at high level.

3.2.3. The Antagonistic Ability of *T. reesci* on *A. flavus*

The antagonistic ability of 10 strains of *T. reesci* on *A. flavus* were showed at Table 4.

Table 4. The antagonistic ability of *T. reesci* on *A. flavus* after 7 days of old fungal culture

No.	Provinces	Strains	PIRG (%)	The antagonistic levels
1		*Tri*.069(2).QX	75.91	++++
2		*Tri*.065(1).QX	64.11	+++
3	Thanh Hoa	*Tri*.004(1).NC	62.00	+++
4		*Tri*.081(1).QX	50.90	++
5		*Tri*.033(1).TG	34.82	+
6		*Tri*.007(1).NĐ	100.00	++++
7	Nghe An	*Tri*.040(2).NL	44.20	+
8		*Tri*.032(1).NL	42.21	+
9	Ha Tinh	*Tri*.127(2).CL	40.21	+
10		*Tri*.100(1).TH	8.21	+

Very high (++++); High (+++); Medium (++); Low (+)

The antagonistic level (20.00%) were very high in 2 strains *Tri*.069(2).QX and *Tri*.007(1).NĐ. There were 2 strains (20.00%) showed with PIRG at high level.

3.2.4. The Antagonistic Ability of *T. hamatum* on *A. flavus*

The antagonistic ability of 20 strains of *T. hamatum* on *A. flavus* showed at Table 5.

Table 5. The antagonistic ability of *T. hamatum* on *A. flavus* after 7 days old fungal culture

No.	Provinces	Strains	PIRG (%)	The antagonistic levels
1		*Tri*.039(1).TG	100.00	++++
2		*Tri*.149(1).TG	91.01	++++
3	Thanh Hoa	*Tri*.008(1).NC	75.21	++++
4		*Tri*.013(4).NC	65.60	+++
5		*Tri*.062(1).QX	50.00	++
6		*Tri*.016(2).NĐ	65.60	+++
7		*Tri*.016(1).NĐ	65.00	+++
8		*Tri*.062(1).QL	61.00	+++
9		*Tri*.020(2).NĐ	60.71	+++
10	Nghe An	*Tri*.063(2).NĐ	62.52	+++
11		*Tri*.023(1).QL	60.00	+++
12		*Tri*.013(2).NĐ	34.25	+
13		*Tri*.022(2).NP	11.00	+
14		*Tri*.009(1).NĐ	9.00	+
15		*Tri*.095(1).TH	85.98	++++
16		*Tri*.092(1).TH	78.00	++++
17	Ha Tinh	*Tri*.118(1).TH	48.61	+++
18		*Tri*.123(1).TH	45.21	+
19		*Tri*.135(1).CL	42.46	+
20		*Tri*.126(1).CL	33.31	+

Very high (++++); High (+++); Medium (++); Low (+)

Among 20 strains of *T. hamatum* that were inoculated, the PIRG of 5 strains (25.00%) were show at very high including *Tri*.039(1).TG, *Tri*.149(1).TG, *Tri*.095(1).TH, *Tri*.092(1).TH and *Tri*.008(1).NC. There were 8 strains (40.00%) with PIRG at high level.

3.2.5. The Antagonistic Ability of *T. virens* on *A. flavus*

The antagonistic ability of 20 strains on *T. virens* and *A. flavus* showed at Table 6.

Table 6. The antagonistic ability of *T. virens* on *A. flavus* after 7 days of old fungal culture

No.	Provinces	Strains	PIRG (%)	The antagonistic levels
1	Thanh Hoa	*Tri*. 014(4).NC	100	++++
2		*Tri*. 100(2).NC	74.32	+++
3		*Tri*. 062(2).QX	70.37	+++
4		*Tri*. 142(1).TG	66.64	+++
5		*Tri*. 003(2).NC	50.90	++
6		*Tri*. 156(2).TG	34.82	+
7		*Tri*. 046(2).NĐ	85.98	++++
8	Nghe An	*Tri*. 022(1).NL	70.00	+++
9		*Tri*. 042(1).NĐ	65.60	+++
10		*Tri*. 035(1).NL	58.80	++
11		*Tri*.046(1).NL	47.60	+
12		*Tri*.006(1).NĐ	44.91	+
13		*Tri*.030(1).NĐ	42.42	+
14	Ha Tinh	*Tri*.057(1).NX	94.51	++++
15		*Tri*.014(2).NX	75.22	++++
16		*Tri*.123(2).TH	62.70	+++
17		*Tri*.010(1).NX	48.61	+
18		*Tri*.128(1).CL	40.21	+
19		*Tri*.059(1).NX	26.27	+
20		*Tri*.101(2).TH	8.21	+

Very high (++++); High (+++); Medium (++); Low (+)

Result showed that, there were four strains (20.00%) that very high antagonistic level and 6 strains (30.00%) at high level.

3.2.6. The Antagonistic Ability of T. pseudokonigii, T. aureoviride, T. viride on A. flavus

Table 7. The antagonistic ability of *T. pseudokonigii, T. aureo viride, T. viride* on *A. flavus* after 7 days of old fungal culture

Species	Strains	PIRG (%)	Provinces	The antagonistic levels
T. pseudokonigii	*Tri*.013(1).NC	99.4	Thanh Hoa	++++
	Tri.014(1).NL	100	Nghe An	++++
	Tri.059(1).NL	54.5	Nghe An	++
	Tri.006(2).NX	100	HaTinh	++++
	Tri.164(1).CL	50.90	Ha Tinh	++
T. aureoviride	*Tri*.039(2).DC	60.4	Nghe An	+++
	Tri.027(2).NĐ	60.7	Nghe An	+++
	Tri.104(1).TH	54.5	Thanh Hoa	++
	Tri.020(1).NX	38.4	Ha Tinh	+
T. viride	*Tri*.123(2).TH	38.4	Thanh Hoa	+

Very high (++++); High (+++); Medium (++); Low (+)

T. pseudokonigii had 3 strains which showed at very high antagonistic level including *Tri*.013(1).NC, *Tri*.014(1).NL, *Tri*.006(2).NX. *T. aureoviride* had 2 strains with the high antagonistic level. The PIRG of *T. viride* was 38.4%, showed at low antagonistic level with *A. flavus.*

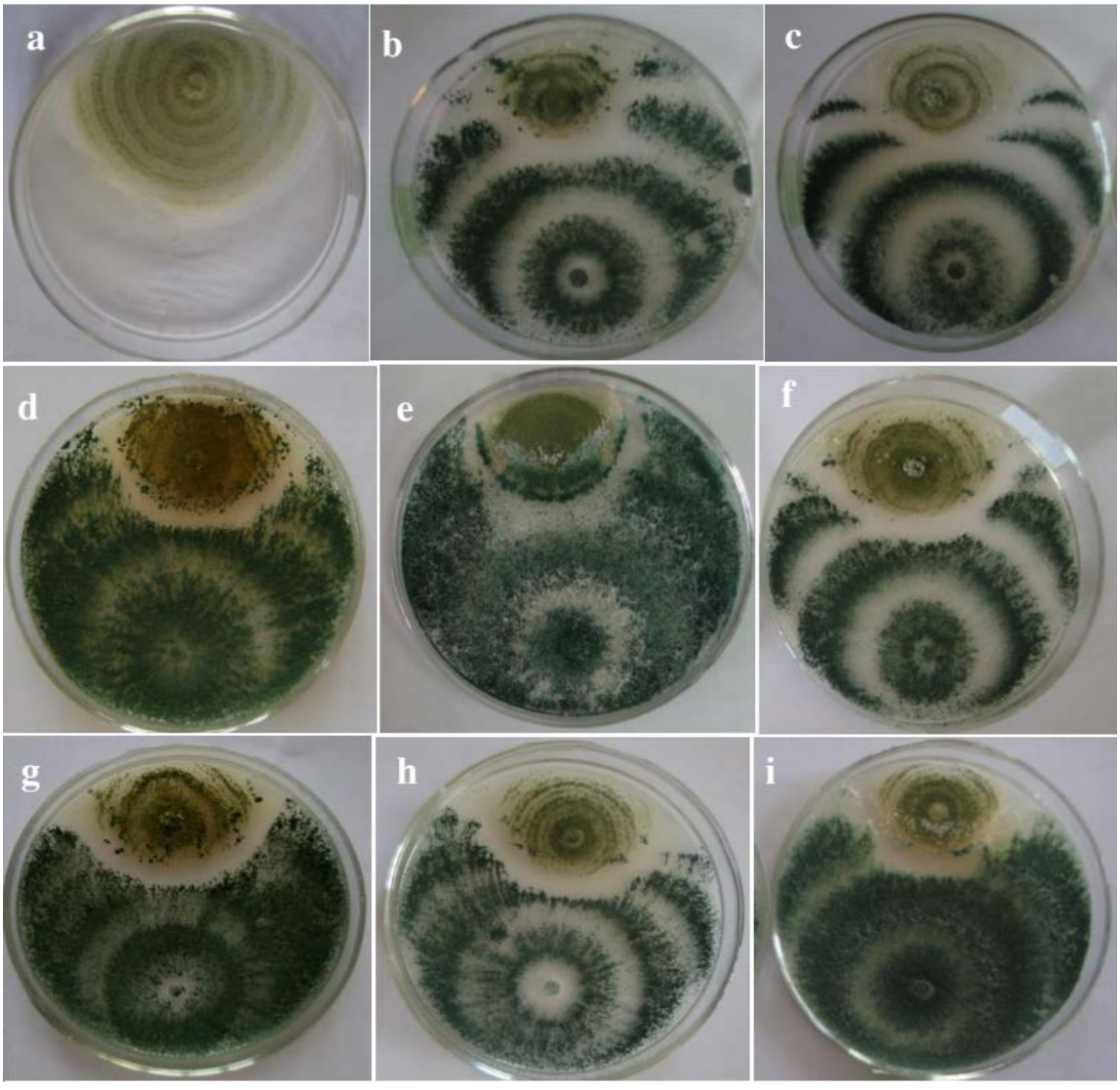

Figure 2. The antagonistic ability of *Trichoderma* spp. on *A. flavus*: **a**. The radius of *A. flavus* mycelium after 7 of inoculation in the control plate, **b-i**: The radius of *A. flavus* mycelium in the dual culture plate, shows overgrowth of *Trichoderma* covering the *A. flavus* colony after 7 days of inoculation in dual culture: *T. harzianum* (*Tri*.011(1).NL, *Tri*.002(2).NC, *Tri*.053(1).TG), *T. atroviride* (*Tri*.005(1)NX, *Tri*.011(1).NC, *Tri*.020(2).NC, *T. hamatum* (*Tri*.092(1).TH), *T. virens* (*Tri*.046(2).NĐ), respectively

4. Conclusion and Recommendations

4.1. Conclusions

(1) Among 1000 soil samples that cultivate peanuts from three provinces Nghe An, Thanh Hoa, Ha Tinh, the appear frequency of *Trichoderma* strains was 44.90%. The appear frequency of *Trichoderma* strains of each province was: Nghe An 44.30%; Thanh Hoa 52.00% and Ha Tinh 39.50%.

(2) Evaluation the antagonistic ability of 99 *Trichoderma* strains on *A. flavus* was carried out by using dual old fungal culture method on PDA medium. There were 28 strains (28.3%) showed at very high antagonistic level. The highest PIRG was 100% included *T. harzianum* (*Tri*.019(4).NC; *Tri*.053(1).TG; *Tri*.011(1).NL; *Tri*.002(2).NX strain), *T. atroviride* (*Tri*.020(2).NC; *Tri*.011(1).NC strain), *T. reesci Tri*.007(1).NĐ, *T. hamatum Tri*.039(1).TG, *T. virens Tri*. 014(4).NC and *T. pseudokonigii Tri*.014(1).NL.

4.2. Recommendations

Using *Trichoderma* strains that have the very high antagonistic level on *Aspergillus flavus* for further research to make bio-products of control *Aspergillus flavus.*

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